73 Detection of antibodies to *Pseudomonas aeruginosa* in oral fluid from patients with Cystic Fibrosis

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Aims: Sensitive and timely detection of early pulmonary infection in children with CF is important as they often do not expectorate sputum. We have investigated the suitability of an oral fluid assay for the detection of antibodies to *Pseudomonas aeruginosa* in order to detect the first antibody response to *P. aeruginosa* in patients.
Methods: We investigated the incidence of A-band LPS in 49 clinical strains of *P. aeruginosa* isolated from CF patients. The detection of antibodies to A-band LPS was assessed (by SDS-PAGE/immunoblotting and ELISA) in oral fluid from 17 CF patients, all of whom were sputum culture-positive for *P. aeruginosa*. Thirteen of the patients also provided serum samples. Additionally, oral fluid from 37 volunteers was examined to assess the specificity of the immunoassay.
Results: Forty-five of the clinical isolates demonstrated LPS consistent with A-band. Antibodies to A-band LPS were detected by immunoblotting in all 17 patients' oral fluids but 10 of the volunteer samples gave weak reactions in immunoblots. However, the ELISA clearly differentiated between the patient and volunteer samples in that 15 of the 17 patient oral fluid samples were positive compared with none of the volunteer samples. All patient serum samples were positive by both methods.
Conclusions: A-band LPS appears to be a suitable antigenic target. We confirm that in addition to conventional serology, A-band antibodies can also be detected in oral fluid from *P. aeruginosa*-infected patients with CF. Further work is required to determine the clinical utility of the assay.

74 Is BPI-ANCA in *Pseudomonas aeruginosa* colonized CF patients strain dependent?

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Autoantibodies against bactericidal/permeability increasing protein (BPI-ANCA) are associated with poor prognosis in CF and so is colonization with *Pseudomonas aeruginosa* (PA). However, the temporal relationship between colonization and the development of the autoantibodies is largely unknown.
BPI-ANCA was measured by ELISA in 245 patients from two CF care units (Lund, Sweden and Copenhagen, Denmark). The patients were divided into age cohorts (ages 12–17, 18–23, 24–29, 30–35 and above 35). Lung transplanted (tpx) patients were excluded. The percentage of patients with serious lung damage, with PA colonization and with positive BPI-ANCA increased with age. In each age group, the percentage of PA colonized patients BPI-ANCA positive patients were strongly associated; so that 58% (17–83%) of the PA colonized patients were also BPI-ANCA positive. In the Swedish patients (n = 121, age 0–52), BPI-ANCA was measured every sixth month for at least two samples. Significant change in BPI-ANCA was rare; in 86% of the patients BPI-ANCA was stable during the study period. Since 1997, seven CF patients have been tpx at Lund CF care unit in whom samples for BPI-ANCA were obtained before and after tpx. All patients had extremely high levels of BPI-ANCA pre-tpx. Post-tpx, all patients had decreased significantly in BPI-ANCA.
These data suggest that BPI-ANCA development occurs early in the course of PA infection in most but not all PA colonized CF patients, and that BPI-ANCA decrease when PA infection is cleared by tpx. We speculate that certain strains of PA are prone to induce BPI-ANCA and that these strains also tend to cause lung damage.

75 Anaerobic survival of *Pseudomonas aeruginosa* requires the Usp-like stress protein PA3309

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Pseudomonas aeruginosa is an opportunistic pathogen causing severe lung infections in patients suffering from cystic fibrosis. The bacterium forms biofilm-like microcolonies embedded in the lung mucus and generates a local anaerobic environment. Survival under these conditions requires nitrate respiration, arginine or pyruvate fermentation for energy generation.
Proteome analysis of pyruvate fermentation revealed increased synthesis of two universal stress proteins (Usp), PA3309 and PA4352. E. coli Usp proteins are involved in resistance to diverse stresses and stationary phase survival, but their exact biological role is still unknown.
A PA3309 knock-out mutant was constructed and phenotypically characterized. This mutant showed none of the typical E. coli usp mutant phenotypes but a severe reduction in anaerobic survival during pyruvate fermentation compared to the *P. aeruginosa* wild type PAO1.
The induction of a transcriptional PA3309 promoter-gfp fusion was monitored in a flow-cell biofilm setup. Promoter activity was detected only in the deeper layers of the biofilm. Furthermore, a transcriptional PA3309 promoter-lacZ fusion is induced in the aerobic and anaerobic stationary phase of planktonic cultures. Anaerobic induction of PA3309 is Anr-dependent; the aerobic stationary phase regulation is currently unknown. To unravel the PA3309 regulation and to examine differences in the expression profile of *P. aeruginosa* PAO1 and the ΔPA3309 mutant during pyruvate fermentation, a transcriptome approach using Affymetrix *P. aeruginosa* Gene Chips is applied.

76 A *Pseudomonas aeruginosa* strain isolated from Cystic Fibrosis lung with strong adherence to human tracheobronchial mucin

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The glycosylation of mucins in the cystic fibrosis (CF) airway is changed. The adherence properties of two *P. aeruginosa* CF clonal variants TB and 892 were examined in comparison with the reference strain PAO1 in adherence assays using glycoconjugate-coated microplates. Bacterial adherence was quantified by CFU. Strain TB was strongly adhering to inanimate and viable surfaces and recognized desialylated glycoconjugates and O-glycosylated oligosaccharides of mucins. Addition of fucose and galactose could compete with binding of TB and 892. Although affinity of strain 892 to complex sugars was weaker than that of TB, the binding capacity of both strains to epithelia was comparable. Analysing the mRNA level of FliD as the prominent mucin adhesin in *P. aeruginosa* did not reveal any differences between the strains TB, 892 and PAO1. However, transcription level of both lectin genes (PA2570, pa1L and PA3361, lecB) were 10- to 80-fold upregulated in TB and 892 compared to PAO1 and two other highly virulent CF isolates CHA and LES. Hence, Pseudomonas lectins may represent the major mucin adhesins of the *P. aeruginosa* TB clone.